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Supporting Information

Ethanol Production by the Hyperthermophilic Archaeon *Pyrococcus furiosus* by Expression of Bacterial Bifunctional Alcohol Dehydrogenases

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Table S1. Amino acid sequence identity (%) among the eight AdhE proteins [a]

	Te	Tx	Gs	Gt	Af	Ct	Tc	Dk
Te	100	97	67	68	68	52	54	65
Tx	97	100	68	68	68	52	54	65
Gs	67	68	100	97	88	51	53	63
Gt	64	68	97	100	89	51	54	63
Af	68	68	88	89	100	51	56	64
Ct	52	52	51	51	51	100	71	47
Tc	54	54	53	54	56	71	100	49
Dk	65	65	63	63	64	47	49	100

[a] Organism abbreviations are as follows: Te, *Thermoanaerobacter ethanolicus* JW200; Tx, *Thermoanaerobacter* sp. X514; Gs, *Geobacillus stearothermophilus* NUB3621; Gt, *Geobacillus thermoglucosidasius* NBRC 107763 Af, *Anoxybacillus flavithermus* WK1; Ct, *Clostridium thermocellum*; Tc, *Thermobrachium celere* DSM 8682; Dk, *Desulfotomaculum kuznetsovii* DSM 6115.

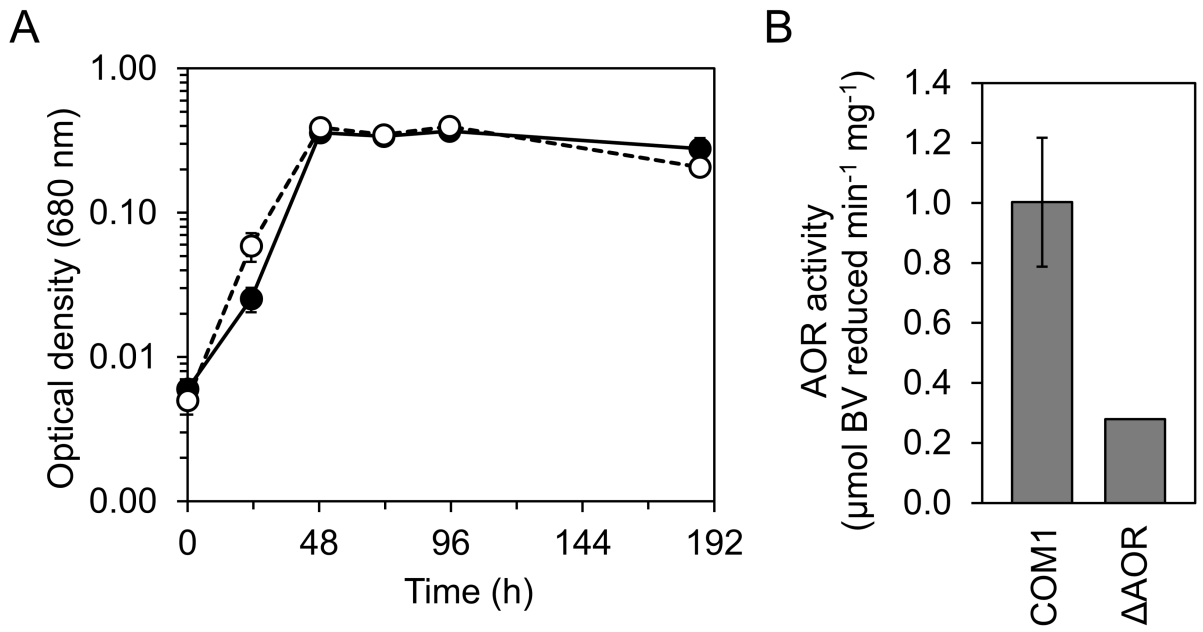

Figure S1. Deletion of *aor* does not affect growth of *P. furiosus* and abolishes 80% of aldehyde oxidoreductase activity. **A.** MW004 (Δ*pyrF*::*pyrF*, black line with closed circles) and MW616 (Δ*pyrF* Δ*aor*::P_{gdh}*pyrF*, dashed line with open circles) strains were grown at 72°C for 72 h and then at 65°C up to 8 d in YM medium containing a total of 2 g L^{-1} yeast extract. Error bars represent S.D., *n* = 3. **B**. Aldehyde oxidoreductase (AOR) activity in cell extracts of COM1 (Δ*pyrF*) and ΔAOR(Δ*pyrF* Δ*aor*).

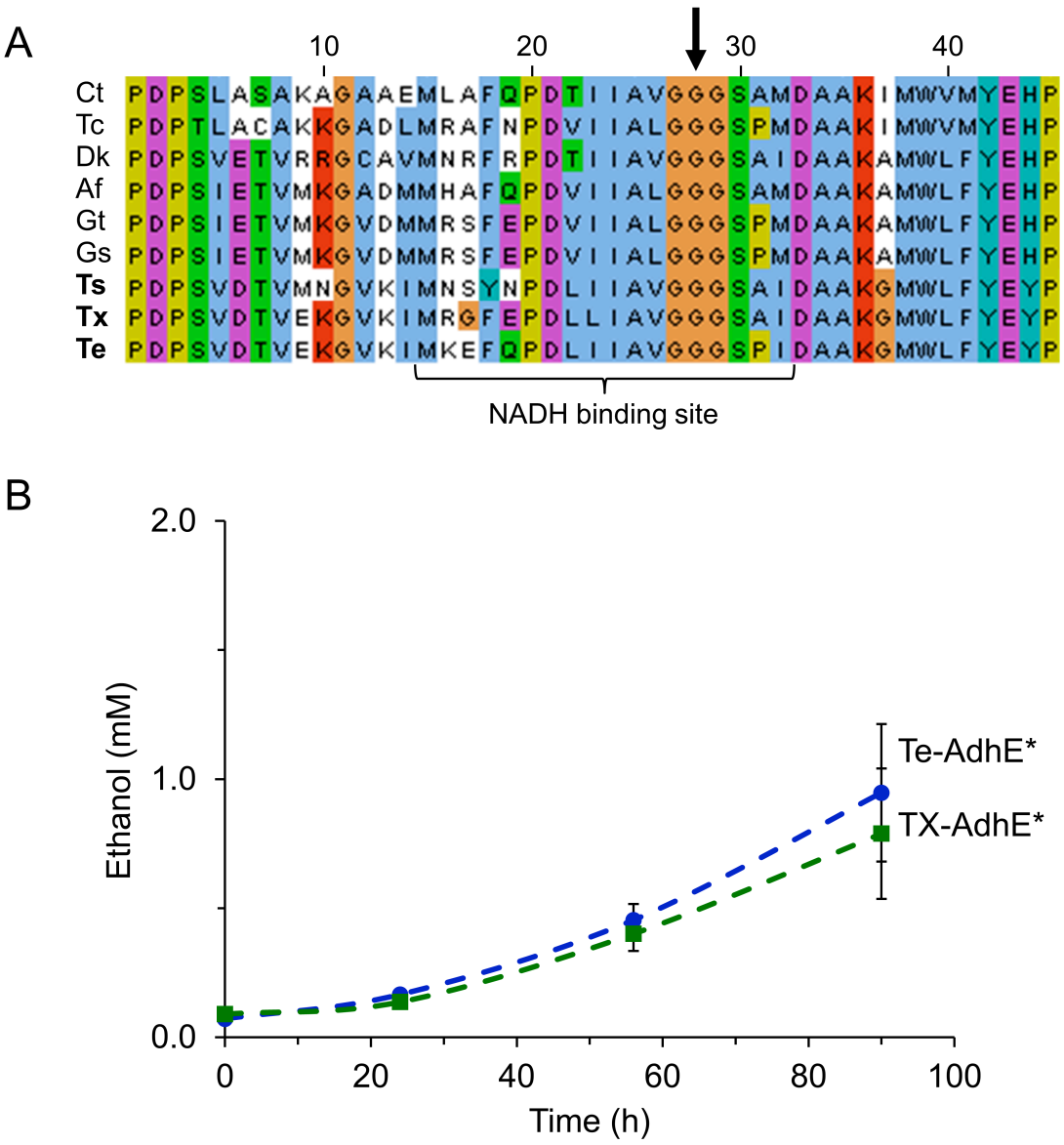

Figure S2. Nucleotide specificity mutation does not improve ethanol yield. **A.** Multiple alignment of ADH domain NADH binding site of selected AdhEs with *T. saccharolyticum* AdhE. Alignment performed using Clustal Omega (Sievers, et al., 2011) and visualized with Jalview (Clamp, et al., 2004). Location of NADH binding site is indicated with a bracket and the position of the G→D mutation (G544D in *T. saccharolyticum*) is indicated with an arrow. Organism abbreviations are as follows: Ct, *Clostridium thermocellum*; Tc, *Thermobrachium celere* DSM 8682; Dk, *Desulfotomaculum kuznetsovii* DSM 6115; Af, *Anoxybacillus flavithermus* WK1; Gt, *Geobacillus thermoglucosidasius* NBRC 107763; Gs, *Geobacillus stearothermophilus* NUB3621; Ts, *T. saccharolyticum*; Tx, *Thermoanaerobacter* sp. X514; Te, *Thermoanaerobacter ethanolicus* JW200. **B.** Ethanol production over time for Te-AdhE and Tx-AdhE, grown in closed serum bottles with 50 mL YM5 medium to mid-log phase at 95°C and then incubated at 65°C for 90 h. Error bars represent S.D., *n* = 3.

References

1 Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., et al. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega, *Molecular Systems Biology* **7**: 539-539.
2 Clamp, M., Cuff, J., Searle, S.M., and Barton, G.J. (2004) The jalview java alignment editor, *Bioinformatics* **20**: 426-427.